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UV inactivation of adenovirus type 41 measured by cell culture mRNA RT-PCR

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Abstract

Adenoviruses are among the most resistant waterborne pathogens to UV disinfection, yet of the 51 serologically distinct human adenoviruses, only a few have been evaluated for their sensitivities to UV irradiation. Human enteric adenoviruses (Ad40 and Ad41) are difficult to cultivate and reliably assay for infectivity, requiring weeks to obtain cytopathogenic effects (CPE). Inoculated cell cultures often deteriorate before the appearance of distinctive CPE making it difficult to obtain reliable and reproducible data regarding UV inactivation. Adenovirus is a double-stranded DNA virus and produces messenger RNA (mRNA) during replication in host cells. The presence of viral mRNA in host cells is definitive evidence of infection. We recently developed a rapid and reliable cell culture-mRNA RT-PCR assay to detect and quantify adenovirus infectivity. Viral mRNA recovered from cell cultures 5–7 days after infection was purified on oligo-dT latex, treated with DNase, and amplified by RT-PCR using the primers specific for a conserved region of the hexon late mRNA transcript. Treatment of approximately 10^4 Ad41 with different doses of 254 nm germicidal UV radiation resulted in a dose-dependent loss of infectivity. As UV doses were increased from 75 to 200 mJ/cm^2, virus survival decreased and no virus infectivity (measured by detectable mRNA) was found at a dose of 225 mJ/cm^2 or higher. Our results using the cell culture mRNA RT-PCR assay indicate that Ad41 is more resistant to UV radiation than in a previous study using a conventional cell culture infectivity assay. Results were more similar to those found for Ad 40 using CPE as a measure of infectivity in another previous study.

Keywords: Enteric adenoviruses; 254 nm UV-radiation; UV disinfection; Infectivity assay

1. Introduction

Germicidal 254 nm UV irradiation has been receiving increased attention as an alternative to chemical disinfection of drinking water and wastewater because UV effectively inactivates cysts and oocysts of protozoan intestinal parasites that are highly resistant to chlorination (Clancy et al., 1998; Linden et al., 2002) and it produces limited toxic disinfection by-products (Haider et al., 2002). Germicidal UV is primarily absorbed by nucleic acids of microorganisms, causing photoproducts such as thymine dimers on the same nucleic acid strand (Harm, 1980). If the damage is not repaired, DNA replication is blocked, leading to

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inactivation of microorganisms. Numerous studies have demonstrated that UV disinfection can effectively inactivate most microorganisms including waterborne pathogens (Linden et al., 2002; Zimmer and Slawson, 2002).

Human enteric adenoviruses, which include the enteric adenovirus serotypes 40 (Ad40) and 41 (Ad41), are important waterborne pathogens. They are the second most commonly identified agent of pediatric gastroenteritis next to rotavirus in many studies (Brandt et al., 1985; McIver et al., 2001) and they have been placed on the United States (US) Environmental Protection Agency (EPA) contaminant candidate list (CCL) (USEPA, 1998). Despite the importance of controlling the enteric adenoviruses in drinking water, only a few studies have been conducted on UV inactivation of enteric adenoviruses (Meng and Gerba, 1996; Thurston-Enriquez et al., 2003). The past studies were performed by observation of infected cells for cytopathogenic effects (CPE) in order to quantify virus infectivity and UV inactivation rates. However, human enteric adenoviruses are difficult to cultivate and reliably assay for infectivity, requiring weeks to obtain CPE. This makes it difficult to obtain reliable and reproducible data on response to UV disinfection because inoculated cell cultures often deteriorate before the appearance of distinctive CPE as evidence of infectivity. We recently developed a new analytical method using cell culture mRNA RT-PCR to reliably measure the infectivity of Ad41(Ko et al., 2003). This method is not dependent upon visual inspection for CPE but rather a definitive molecular test for presence or absence of mRNA in infected cells. The objective of this study was to determine 254 nm UV inactivation kinetics of enteric Ad41 using the recently developed cell culture-mRNA RT-PCR for virus infectivity assay. UV inactivation of Ad41 was compared to that of the widely used virus biodosimeter of UV disinfection, male-specific (F+) RNA coliphage MS2.

2. Materials and methods

2.1. MS2 assay and stock

Bacteriophage MS2 (ATCC # 15597-B1) was grown and assayed by single agar layer method using *Escherichia coli* C3000 (ATCC # 15597) (USEPA, 2001). The virus was purified from infected cell lysates of double agar layer plaque assay plates with confluent lysis by extracting it with an equal volume of chloroform, centrifuging it at 4000*g* for 30 min, and recovering the supernatant fluid. The resulting supernatant was recovered as virus stock and stored frozen in aliquots at −80 °C for use in experiments. The MS2 stock titer was approximately 10^9 plaque-forming units (PFU)/ml.

2.2. Ad41 culture and stock

Ad41 (ATCC#: VR-930) was obtained from ATCC, and cultured on human embryonic kidney (HEK) 293 cells (ATCC#: CRL-1573) in Eagle's Minimal Essential Medium (MEM) containing 2% fetal bovine calf serum. The viruses were inoculated and cultivated on confluent 293 cell monolayer for 2 weeks. Infected cells were frozen and thawed 3 times at −20 °C to release the viruses, mixed with an equal volume of chloroform, and then centrifuged at 2500*g* for 15 min. The upper aqueous layer was recovered, stored frozen at −80 °C, and then used as the stock for UV disinfection experiments. The cell culture infectivity titer of Ad41 was estimated by quantal endpoint assay employing 10-fold dilution series and 4 replicate cultures per dilution in 293 cells to compute the most probable number (MPN) based on amplification of viral DNA. This was the basis for determining infectivity titers because of variable titers based on visual inspection for the appearance of CPE in cell culture. The Ad41 stock titer was approximately 5×10^5 infectious unit/ml.

2.3. Ads-specific primers for cell culture mRNA RT-PCR

Ads-specific primers (Hex1/Hex2) were used to detect hexon gene sequences conserved among all 51 Ads serotypes, as described in previous studies (Ko et al., 2003; Xu et al., 2000), yielding an amplicon of 482 bp DNA amplified by Ad group-specific primers (Hex1/Hex2) was subjected to nested PCR (Hex1/Hex3), with a resulting amplicon of 443 bp. The sequences of Hex1, Hex2, and Hex3 primers are 5′-TTCCCCATGGCICA(CT)AACAC-3′, 5′-CCCTGGTA(GT)CC(AG)AT(AG)TTGTA-3′, and 5′-AGGAACCA(AG)TC(CT)TT(AG)GTCAT-3′, respectively. The sensitivity of the primers based on the lower limit (dilution endpoint titration) of DNA amplification relative to virus infectivity was evaluated in a previous study (Ko et al., 2003).

2.4. Virus infection and mRNA purification

Newly confluent cell culture monolayers were inoculated with Ad41 suspended in phosphate-buffered saline (PBS) and incubated with periodic gentle mixing for 1 h at 37 °C. After the inoculum was removed, 10 ml of MEM containing 2% fetal bovine serum was added to the inoculated cell monolayer for culture at 37 °C, typically for 5–7 days. To purify mRNA from the inoculated 293 cell cultures the cell monolayer was first washed with 5 ml of PBS, and then treated with 5 ml of 0.25% trypsin (Sigma, St. Louis, MO) in PBS for 5 min. The trypsinized cells were transferred to a polypropylene centrifuge tube, harvested by centrifugation at 300*g* for 5 min, and the supernatant was discarded. The recovered

cell pellet was lysed by 3% β-mercaptoethanol and guanidium isothiocynate (GITC), and then homogenized by QIAshredder (QIAGEN, Valencia, CA). The mRNA in cell lysates was purified by oligo-dT latex following the standard protocol of the Oligotex direct mRNA kit (QIAGEN, Valencia, CA) to give a final volume of 60 μl. To remove any contaminant DNA, 10 μl of mRNA was incubated for 15 min with 2 μl of RQ1 RNase-free DNase, 2 μl of DNase buffer (Promega, Madison, WI), and 1 μl of RNase inhibitor (Promega) in a 20-μl volume. Residual DNase was inactivated by DNase stopping solution (Promega). An adenovirus PCR reaction was performed to check for any residual contaminating DNA, and the absence of Ad DNA was confirmed by electrophoresis on a 2% agarose gel followed by ethidium bromide staining. Viral DNA was extracted using the QIAamp viral kit following the standard protocol (QIAGEN, Valencia, CA).

2.5. RT-PCR and hemi-nested PCR conditions

The RT reaction was performed in 15 μl volumes using 10 × PCR buffer (pH = 8.3), containing 3 μM of primer, 3 mM $MgCl_2$, 1 mM nucleotide mix, 5 U of AMV-RT (Promega), and 20 U of RNase inhibitor (Promega) at 42 °C for 1 h, followed by inactivation of RTase at 94 °C for 2 min. PCR was performed in 50 μl volumes containing 45 μl of reaction mixture (10 × PCR buffer [Promega]: 10 mM Tris-HCl [pH 9.0], 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM each nucleotide, 0.6 μM each primer, 2.5 U of Taq DNA polymerase [Invitrogen, Carlsbad, CA]) per reaction and 5 μl of RT reaction of sample. Amplification reactions were carried out in a thermocycler (model#: PTC-200; MJ Research, Watertown, MA) with preliminary denaturation for 5 min at 94 °C, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, primer extension at 72 °C for 2 min, and final extension at 72 °C for 5 min. In all mRNA RT-PCR reactions, RT-PCR without RTase also was performed in order to prove no DNA contamination in mRNA extracts. Hemi-nested PCR was performed in 50 μl volumes containing 49 μl of reaction mixture (10 × PCR buffer [Promega]: 10 mM Tris-HCl [pH 9.0], 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM each nucleotide, 0.6 μM each primer, 2.5 U of Taq DNA polymerase [Invitrogen]) per reaction and 1 μl of either undiluted or 10-fold diluted RT-PCR reaction sample. Amplification reactions were carried out in a thermocycler with preliminary denaturation for 5 min at 94 °C, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, primer extension at 72 °C for 2 min and then final extension at 72 °C for 5 min. Ten microliters of each reaction product was separated (1 h, 120 V) on 2% agarose gels, stained with ethidium bromide, and visualized by UV transillumination.

2.6. UV disinfection

A collimated (quasi parallel) beam UV apparatus containing two 15–W, low-pressure mercury vapor germicidal lamps emitting nearly monochromatic UV radiation at 254 nm was used. The emitted UV light was directed through a circular opening to provide incident radiation to the surface of the test suspension in a 60 × 15 mm cell culture Petri dish. The UV irradiance was measured with a radiometer (Model IL500; International Light, Inc., Newburyport, MA) that had been factory calibrated, traceable to National Institute of Standard and Technology standards, prior to the study. The measured incident irradiance at the surface of the test liquid was corrected for any nonhomogeneity of irradiation across the surface area of the Petri dish to provide the value for average incident irradiance. Approximately 10^4 IU of Ad41 and 10^8 PFU of MS2 were suspended in 5 ml PBS, and exposed to 254 nm UV at room temperature. The average irradiance in the mixed suspension was determined mathematically by the Beer–Lambert law over the sample depth, accounting for UV absorbance of the test suspension and incident average irradiance (Morowitz, 1950). In our study, the depth of viral suspension was 0.4 cm, and 254 nm UV absorbance of the viral suspension was approximately 0.053/cm. The UV exposure doses for Ad41 and MS2 were 0, 75, 150, 225 and 300 mJ/cm^2 or 0, 74, 147, 201 mJ/cm^2, respectively. The UV exposure doses were calculated from the products of exposure time and calculated UV irradiance. Virus titers were estimated by the previously described MPN method based on positive and negative mRNA RT-PCR results from 1–3 replicate cell culture flasks per dilution for serially diluted samples.

2.7. UV inactivation kinetics

First-order inactivation kinetics was assumed for UV inactivation of tested microorganisms. The data were fitted by regression analysis of the following equation: $\log_{10}(N_t/N_o) = -kIt$, where N_0 is the concentration of infectious virus at time = 0, N_t is the concentration of infectious virus at time = t, k is the inactivation rate or slope of $\log_{10}$ virus infectivity reduction per UV dose, I is the UV irradiance (μW/cm^2), and t is the exposure time (s). The parameter $-\log_{10}$ of the survival ratio (N_t/N_0) versus dose for each experiment was estimated by linear regression analysis for each virus by using STATA (College Station, TX). Intercepts of first order inactivation kinetics were set to 0 at UV dose = 0.

3. Results

Table 1 and Fig. 1 show the inactivation kinetics of Ad41 and MS2 as a function of UV dose. Unlike MS2,

Table 1
Inactivation of Ad41 and MS2 in buffered water by 254 nm UV radiation

Microorganism	UV dose (mJ/cm^2)	Log_{10} reduction					Mean ± SD
		Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	
Adenovirus 41[a]	75	2.0	1.0	n/a	n/a	0.7	1.2 ± 0.7
	150	3.0	3.0	3.3	2.6	2.5	2.9 ± 0.3
	175	n/a	n/a	3.8	2.7	2.5	3.0 ± 0.7
	200	n/a	n/a	3.8	>3.0	3.4	3.6 ± 0.3
	225	>3.0	>3.0	>3.8	n/a	n/a	n/a
	300	>3.0	>3.0	>3.8	n/a	n/a	n/a
MS2[b]	74	3.5	3.5	n/a	n/a	n/a	3.5
	147	5.0	5.4	n/a	n/a	n/a	5.2
	201	6.2	7.6	n/a	n/a	n/a	6.9

[a]Analyzed by mRNA RT-PCR.
[b]Analyzed by single agar layer (SAL) plaque assay method.

whose maximum log_{10} reduction is >6, the maximum detectable log_{10} reduction of Ad 41 is only 3.8 due to the lower titer of Ad41 viral stock. Ad41 was very resistant to UV, with a small fraction of the initial virus surviving even at a UV dose of 200 mJ/cm^2 (3.6 log_{10} reduction), and the detection limit (>3.8 log_{10} reduction) was reached at the UV dose of 225 mJ/cm^2 or higher. The reduction of Ad41 was approximately first order, with no evidence of tailing or flattening of inactivation curve, and an inactivation rate constant estimated to be 0.018 ± 0.002 (mean ± SE) cm^2/mJ and an R^2 of 0.99. The reduction of MS2 by UV irradiation was considerably more extensive and rapid than that of Ad41. MS2 inactivation was approximately first order and the inactivation rate constant was estimated to be 0.034 ± 0.002 (mean ± SE) cm^2/mJ, with an R^2 of 0.99. The MS2 inactivation rate was similar to previously reported results (Linden et al., 2003; Thurston-Enriquez et al., 2003).

Table 2 shows a comparison of past studies and this study on monochromatic 254 nm wavelength UV disinfection of enteric adenoviruses and MS2. For adenovirus 40, the study by Thurston-Enriquez et al. (2003) shows higher UV resistance than the one by Meng and Gerba (1996). In both studies adenovirus infectivity was analyzed by conventional cell culture methods based on CPE. Our study using cell culture mRNA RT-PCR for virus infectivity found Ad41 to have much higher resistance to monochromatic 254 nm UV than the study by Meng and Gerba using CPE to score and quantify infectivity. The UV resistance of Ad41 in our study is very close to that of Ad40 in the study by Thurston-Enriquez et al. (2003). The UV resistance of MS2 in our study is very close to that reported by Thurston-Enriquez et al. but somewhat higher than that reported by Meng and Gerba. All of the studies indicated that the virus inactivation kinetics of UV disinfection was reasonably well described by a first-order kinetic model. Hence, the reported differences in the extent of UV inactivation of adenoviruses are not explained by declining inactivation rates or tailing.

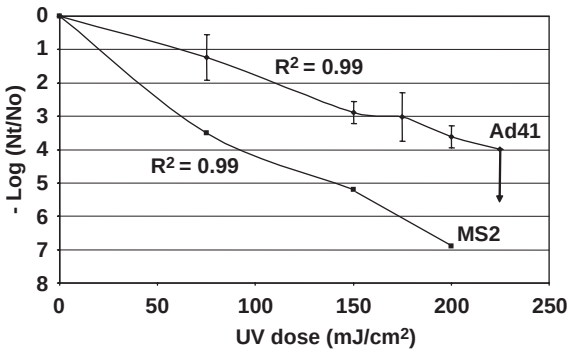

Fig. 1. Kinetics of inactivation of adenovirus 41 and MS2 by 254 nm UV. R^2 is a correlation coefficient and was obtained from a linear regression. ↓, Detection limit.

4. Discussion

There have been only a few studies on UV inactivation of enteric adenoviruses in spite of the importance of these viruses as waterborne pathogens (Meng and Gerba, 1996; Thurston-Enriquez et al., 2003). Furthermore, the results from these two studies are not in agreement. A likely reason for the limited number of studies and the inconsistent results in the past is the lack of a reliable method to quantify the infectivity of enteric adenoviruses. To overcome the difficulties of estimating

Table 2
Summary of data from past and our studies on 254 nm UV inactivation rates of MS2 and enteric adenoviruses (Ad40 and Ad41)

Microorganism	Meng and Gerba (1996)			Thurston-Enriquez et al. (2003)			Our study		
Microorganism	Ad40	Ad41	MS2	Ad40	Ad40	MS2	Ad41		MS2
Suspending media[a]	DW or tap water	DW or tap water	DW or tap water	BDF	Ground	BDF	PBS		PBS
Inactivation constant (cm^2/mJ)	0.032	0.037	0.058	0.0171 ± 0.0013	0.0201 ± 0.0012	0.0310 ± 0.0013	0.0180 ± 0.002		0.034 ± 0.002
R^2	0.99	0.98	0.99	0.88	0.92	0.97	0.98		0.99
UV dose (mJ/cm^2) for 1 log inactivation	30	24	14	50	53	23	56 (53–58 [95% CI])		29
UV dose (mJ/cm^2) for 4 log inactivation[b]	124	112	65	226	203	119	222 (213–232 [95% CI])		118

[a]DW: distilled water; tap water: activated carbon dechlorinated tap water; BDF: buffered-demand-free (pH = 7.0); ground: dechlorinated groundwater (pH = 8.0); PBS: phosphate buffered saline (pH = 7.4).
[b]Estimated dose based on the linear regression.

enteric adenovirus infectivity titers based on scoring for the appearance of CPE, we characterized monochromatic 254 nm UV inactivation kinetics of enteric adenovirus Ad41 using a recently developed and reliable molecular method based on the appearance of virus-specific mRNA in inoculated, infected cell cultures (Ko et al., 2003). The results of these studies indicated that 1, 2, 3 and 4 $\log_{10}$ (90%, 99%, 99.9% and 99.99%) reductions of adenoviruses required UV doses of about 56, 111, 167 and 222 mJ/cm^2, respectively.

A comparison between the results of this study and the past studies on inactivation of enteric adenoviruses by monochromatic 254 nm UV (Table 2) shows significantly higher resistance of Ad41 to 254 nm UV than reported in the past study by Meng and Gerba (1996). Ad41 inactivation of 90% (1 $\log_{10}$) and 99.99% (4 $\log_{10}$) was achieved at estimated UV doses of 56 and 222 mJ/cm^2, respectively, in our study compared to 30 and 124 mJ/cm^2, respectively, in the previous study. It is noteworthy that the extent of UV inactivation of Ad40 estimated by Thurston-Enriquez et al. (2003) was not significantly different from that of Ad41 in our present study. The reasons for the apparent differences in the responses of Ads 40 and 41 to UV radiation are unclear. It could be due to (1) real differences in the susceptibilities of the two viruses to UV radiation, (2) variability of the infectivity assays of the two viruses based on scoring for the appearance of CPE, (3) different viral stock preparation such as repeated free thawing or chloroform extraction, etc., (4) different experimental conditions such as UV exposure setup, UV dosimetry measurements, pH and ionic strength of suspending media, or (5) any combination described above. Variability of experimental conditions in UV disinfection studies is a recognized problem that has led to calls for standardization of procedures and the use of internal controls and benchmarks (Bolton and Linden, 2003). In addition to standardization of UV exposure and dosimetry, the use of a consistent infectivity assay for both Ad40 and Ad41 based on the detection of a virus-specific product such as viral mRNA in infected cell cultures would make it possible to determine if the previously observed differences in response to UV irradiation are real or a consequence of variable and unreliable infectivity assays, UV exposure conditions and dosimetry or other reasons.

Previous studies also showed that different adenovirus serotypes had considerably different UV susceptibilities (Gerba et al., 2002; Meng and Gerba, 1996; Thurston-Enriquez et al., 2003). For achieving a 4 $\log_{10}$ reduction by monochromatic 254 nm UV, a dose of 160 mJ/cm^2 was required for adenovirus serotype 2 (Ad2) (Gerba et al., 2002), while doses of 203–226 mJ/cm^2 were required for Ad40 in one study (Thurston-Enriquez et al., 2003) and doses of 112–124 mJ/cm^2 were required for Ad40 and Ad41 in another study (Meng and Gerba, 1996).

Our study using cell culture mRNA RT-PCR for infectivity assay suggests that Ad41 is more resistant to UV than Ad2 and than Ad40 and Ad41 reported in one previous study (Meng and Gerba, 1996). It should be noted that, unlike enteric adenoviruses, Ad2 produces very clear CPE and the conventional cell culture infectivity assay method is considered more reliable. Differences in observed UV resistance among different adenovirus serotypes may be caused by differences in structure, genetic variation, different viral assay methods, and different experimental procedures for preparing and disinfecting the tested viruses. In the future, UV disinfection of Ad40 and other adenoviruses needs to be quantified using cell culture-mRNA RT-PCR using similar experimental conditions as described here for Ad41 in order to determine if there is any serotype difference in UV susceptibility among Ad40, Ad41 and other adenoviruses.

Repeated freeze-thawing was speculated to be the reason for inconsistent results in the past studies, suggesting that it causes adenoviruses to become more sensitive to UV (Gerba et al., 2002; Thurston-Enriquez et al., 2003). Our Ad 41 viral stock was prepared by repeated freezing and thawing at least 3 times. However, our results showed that Ad41 was more resistant to UV than reported in previous studies. Our results suggest that either repeated freeze-thawing did not affect the UV susceptibility of Ad41, or our mRNA RT-PCR method to detect infectious viruses is much more sensitive than conventional CPE-based cell culture methods used in the previous studies, or a combination of both.

The role of different virus infectivity assay procedures as the basis for differences in virus inactivation by disinfection processes also has been previously reported for the chemical disinfectant free chlorine. In a previous study based on a cell culture infectivity assay scored by CPE, poliovirus was completely inactivated by a 0.5 mg/l dose of free chlorine after 2 min (Blackmer et al., 2000). However, integrated cell culture-PCR detected chlorinated viruses for up to 8 min of exposure and 10 min were required for complete inactivation. Thus, chlorine disinfection of poliovirus based on detecting viral nucleic acid in infected cell cultures required a 5-fold longer contact time than was previously reported based on detecting viral CPE. Therefore, discrepancies in the kinetics and extent of virus inactivation by disinfection processes based on CPE as opposed to detection of virus-specific macromolecules indicative of infectivity may be a more general problem that deserves further investigation.

There is the need for caution in comparing the adenovirus UV inactivation results of this study to those of previous studies. In each study, the experimental conditions were slightly different. For example, different suspending media (e.g., sterile distilled water or activated-carbon dechlorinated tap water, treated groundwater, or buffered-demand-free water) were used in previous studies while PBS (pH = 7.4) was used in our study. Although UV absorbance of suspending media was measured and adjusted in all of studies using the Beer–Lambert law (Morowitz, 1950), other factors such as the properties of the UV dosimetry apparatus, the reliability of UV dose measurement, the ionic strength and pH of the suspending medium, and the extent of virus aggregation or particle association could have affected UV inactivation rates of enteric adenoviruses. For better comparison, UV inactivation rates should be measured side-by-side using both mRNA RT-PCR and conventional CPE detection with the same experimental conditions.

Male-specific RNA coliphage MS2 is the most commonly used microorganism as a UV biodosimeter, and the UV inactivation rate of MS2 has been well characterized in many previous studies (Linden et al., 2003). In our study, we characterized the UV inactivation of MS2 for comparison between our study and other studies. The UV inactivation rate of MS2 in our study was comparable to that in many but not all previous studies: $0.034 \pm 0.002\,cm^2/mJ$ in our study, $0.0310 \pm 0.0013\,cm^2/mJ$ in the study by Thurston-Enriquez et al. (2003), but somewhat different from the rate of $0.058\,cm^2/mJ$ reported by Meng and Gerba (1996). These results suggest that our experimental conditions were very close to those of the study by Thurston-Enriquez but somewhat different from a study by Meng and Gerba. Further studies are needed for better understanding the factors that have contributed to the reported differences in MS2 inactivation by LP UV and how to best account for and control these factors.

It should be also noted that adenoviruses use the host cell enzymes to repair their own DNA damage caused by UV. All past studies of other investigators on UV disinfection of adenoviruses used PLC/PRF5 cells while our study used 293 cells instead. Therefore, it is possible that using different types of cell lines may lead to different UV susceptibilities of the same adenovirus due to the extent of and ability to achieve intracellular repair of UV-induced DNA damage. In the future, the effect of different types of cell lines on UV susceptibility needs to be examined, especially with respect to DNA repair of UV-induced DNA damage. Furthermore, the extent of intracellular repair of UV-induced DNA damage in cell cultures may be different than that occurring in human hosts.

Such differences in the extent of DNA repair of UV-induced damage have important implications for UV disinfection practice in relation to drinking water risks from adenoviruses.

5. Conclusion

UV resistance of enteric adenovirus serotype 41 was characterized by using a recently developed mRNA

RT-PCR method. Our study indicated that enteric adenovirus Ad41 was very resistant to 254 nm germicidal UV. Approximately 222 mJ/cm^2 of UV dose would be required for 4 log_{10} inactivation with our experimental conditions, which was significantly higher than previously reported using a conventional CPE-based cell culture infectivity assay method. This is a considerably higher dose than currently employed or proposed for either water or wastewater disinfection by UV radiation. The extent to which differences in experimental conditions and in the extent of DNA repair of UV-induced damage in adenoviruses are responsible for the observed differences in UV inactivation are uncertain and they need further investigation.

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